



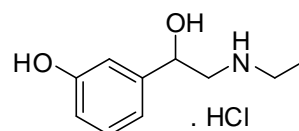
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA M880b: Etilefrine hydrochloride

Report ID: M880b.2019.01

Chemical Formula: C<sub>10</sub>H<sub>15</sub>NO<sub>2</sub>.HCl

Molecular Weight: 217.7 g/mol (HCl), 181.2 g/mol (base)



### Certified value

Batch No.	CAS No.	Purity (mass fraction)
12-D-01	943-17-9 (HCl) 709-55-7 (base)	99.8 ± 1.3%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

**IUPAC name:** 3-[2-(ethylamino)-1-hydroxyethyl]phenol hydrochloride

**Expiration of certification:** The property values are valid till 4 April 2024, i.e. five years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

**Description:** Off-white solid sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

**Intended use:** This certified reference material is suitable for use as a primary calibrator.

**Instructions for use:** Equilibrate the bottled material to room temperature before opening.

**Recommended storage:** When not in use this material should be stored at or below 25 °C in a closed container in a dry, dark area.

**Metrological traceability:** The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%.

**Stability:** In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years. The long-term stability of the compound in solution has not been examined.

**Homogeneity assessment:** The homogeneity of the material was assessed using purity assay by HPLC with UV detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

**Safety:** Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,  
Team Leader,  
Chemical Reference Materials, NMI.  
18 April 2019

This report supersedes any issued prior to 18 April 2019

**NATA logo notice:** Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

**Legal notice:** Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

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## Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100 \% - I_{\text{ORG}}) \times (100 \% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

$I_{\text{ORG}}$  = Organic impurities of related structure,  $I_{\text{VOL}}$  = volatile impurities,  $I_{\text{NVR}}$  = non-volatile residue.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler
	Column:	Ascentis C-18, 2.7 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Water/methanol (60:40) 1% Formic acid was present in the aqueous phase
	Flow rate:	0.2 mL/min
	Detector:	Waters PDA 2998 operating at 273.8 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.98%, s = 0.004% (10 sub samples in duplicate, January 2012)
	Re-analysis:	Mean = 99.99%, s = 0.00% (5 sub samples in duplicate, January 2013)
	Re-analysis:	Mean = 99.96%, s = 0.00% (7 sub samples in duplicate, January 2016)
	Re-analysis:	Mean = 99.99%, s = 0.002% (5 sub samples in duplicate, April 2019)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (January 2012, December 2012, January 2016) Moisture content 0.2% mass fraction (April 2019)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (January 2012)

### Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Positive ion mode, direct infusion at 10 $\mu$ L/min
	Ionisation:	ESI spray voltage at 3.5 kV positive ion
	EM voltage:	650 V
	Cone voltage:	5 V
	Peak:	181.9 (M+H <sup>+</sup> ) <i>m/z</i>
HS-GC-MS:	I Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 $\mu$ m
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Acetone
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 $\text{cm}^{-1}$ , KBr powder
	Peaks:	3552, 3319, 3213, 3095, 2991, 2802, 2728, 2522, 2448, 2303, 1589, 1476, 1308, 1220, 1164, 1065, 933, 837, 793, 699, 602, 545, 475 $\text{cm}^{-1}$
<sup>1</sup> H NMR:	Instrument:	Bruker Avance-III-400
	Field strength:	400 MHz
	Solvent:	D <sub>2</sub> O (4.79 ppm)
	Spectral data:	$\delta$ 1.31 (3H, t, <i>J</i> = 7.2 Hz), 3.16 (2H, q, <i>J</i> = 7.3 Hz), 3.25 (1H, dd, <i>J</i> = 9.1, 13.0 Hz), 3.31 (1H, dd, <i>J</i> = 3.9, 13.0 Hz), 5.00 (1H, dd, <i>J</i> = 3.9, 9.0 Hz), 6.89 (1H, m), 6.94 (1H, m), 6.99 (1H, m), 7.34 (1H, d, <i>J</i> = 7.9 Hz) ppm Acetone estimated at 0.04% mass fraction was observed in the <sup>1</sup> H NMR
<sup>13</sup> C NMR:	Instrument:	Bruker Avance-III-400
	Field strength:	100 MHz
	Solvent:	D <sub>2</sub> O
	Spectral data:	$\delta$ 10.3, 43.1, 52.5, 68.7, 112.7, 115.5, 117.9, 130.4, 141.5, 155.9 ppm
Melting point:		118-122 °C
Microanalysis:	Found:	C = 55.4%; H = 7.6%; N = 6.5% (January, 2012)
	Calc:	C = 55.2%; H = 7.4%; N = 6.4% (Calculated for C <sub>10</sub> H <sub>15</sub> NO <sub>2</sub> .HCl)